View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector



FOXP2 variant of modern humans was shared with Neandertals. Curr. Biol. *17*, 1908–1912.
2. Enard, W., Przeworski, M., Fisher, S.E., Lai, C.S.L., Wiebe, V., Kitano, T., Monaco, A.P., and Pääbo, S. (2002). Molecular evolution of FOXP2, a gene involved in speech and language. Nature *418*, 869–872.
3. Lai, C.S.L., Fisher, S.E., Hurst, J.A., Vargha-Khadem, F., and Monaco, A.P. (2001). A forkhead-domain gene is mutated in a severe speech and language disorder. Nature *413*, 519–523.
4. d'Errico, F. (2003). The invisible frontier: A multispecies model for the origin of behavioral modernity. Evol. Anthropol. *12*, 188–202.
5. Grün, R., and Stringer, C. (2000). Tabun revisited: revised ESR chronology and new ESR and U-series analyses of dental material from Tabun C1. J. Hum. Evol. *39*, 601–612.
6. d'Errico, F., and Soressi, M. (2002). Systematic use of manganese pigment by the Pech-de-l'Azé Neandertals: implications for the origin of behavioral modernity. J. Hum. Evol. *42*, A13.
7. Hovers, E., Ilani, S., Bar-Yosef, O., and Vandermeersch, B. (2003). An early case of color symbolism. Ochre use by modern humans in Qafzeh Cave. Curr. Anthropol. *44*, 491–522.
8. Barham, L.S. (2002). Systematic pigment use in the Middle Pleistocene of south-central Africa. Curr. Anthropol. *31*, 181–190.
9. Marshack, A. (1976). Some implications of the Paleolithic symbolic evidence for the origin of language. Curr. Anthropol. *17*, 274–282.
10. Heim, J.L. (1976). L'homme fossile de La Ferrassie. Arch. Inst. Paléontol. Hum. *35*, 1–331.
11. Marshack, A. (1996). A Middle Paleolithic symbolic composition from the Golan Heights: The earliest known depictive image. Curr. Anthropol. *37*, 357–365.
12. Davis, S., (1974). Incised bones from the Mousterian of Kebara Cave (Mount Carmel) and the Aurignacian of Ha-yonim Cave (western Galilee), Israel. Paléorient *2*, 181–182.
13. Henshilwood, C.S., d'Errico, F., Yates, R., Jacobs, Z., Tribolo, C., Duller, G.A.T., Mercier, N., Sealy, J.C., Valladas, H., Watts, I., *et al*. (2002). Emergence of modern human behavior: Middle Stone Age engravings from South Africa. Science *295*, 1279–1280.
14. d'Errico, F., Henshilwood, C., Vanhaeren, M., and van Niekerke, K. (2005). *Nassarius kraussianus* shell beads from Blombos Cave: evidence for symbolic behaviour in the Middle Stone Age. J. Hum. Evol. *48*, 3–24.
15. Bouzouggar, A., Barton, N., Vanhaeren, M., d'Errico, F., Collcutt, S., Higham, T., Hodge, E., Parfitt, S., Rhodes, E., Schwenninger, J.L., *et al*. (2007). 82,000-year-old shell beads from north Africa and implications for the origins of modern human behavior. Proc. Natl. Acad. Sci. USA *104*, 9964–9969.
16. Vanhaeren, M., d'Errico, F., Stringer, C., James, S.L., Todd, J.A., and Mienis, H.K. (2006). Middle Paleolithic shell beads in Israel and Algeria. Science *312*, 1785–1788.
17. Wetzel, R., and Bosinski, G. (1969). Die Bocksteinschmiede in Lonetal (Markung Rammingen, Kreis Ulm). Veröffentlich. statt. Amt. Denkmal. Stuttgart. 15(A).
18. Trinkaus, E. (2005). Early modern humans. Annu. Rev. Anthropol. *34*, 207–230.
19. Ruff, C.B., Trinkaus, E., and Holliday, T.W. (1997). Body mass and encephalization in Pleistocene *Homo*. Nature *387*, 173–176.

Department of Anthropology, Campus Box 1114, Washington University, St. Louis, Missouri 63130, USA.
E-mail: trinkaus@artsci.wustl.edu

DOI: 10.1016/j.cub.2007.09.055

# Germlines: Argonautes Go Full Cycle

**Specific Argonaute proteins and their small RNA targets are important in animal germline development. Although plants strictly do not have germlines and form their gametes from gametophytes, there is now evidence that reproductive Argonautes play equally important roles in plants.**

**Robert Grant-Downton and Hugh Dickinson**

Reproductive development in plants and animals might seem, on superficial consideration, to have little in common. In most animals, the lineage of cells giving rise to the gametes —the 'germline' — is specified and segregated from the somatic cell lineages early in development. Plants, in contrast, do not define or segregate such cell lineages at a pre-determined time in their development. Given a continual supply of undifferentiated cells with 'stem' properties generated by the shoot meristems, plants are able to switch gene expression programmes so as to produce reproductive cell lines — rather than more somatic cells. Plants and animals also differ in the developmental programme that leads to gametes, for in animals the haploid products of meiosis immediately differentiate to form the gametes, while in plants the route to gametogenesis is not so direct. Instead, the haploid products of meiosis undergo further mitotic divisions to form a haploid generation called the gametophyte — which generates the gametes (eggs and sperm, as in animals) (see Figure 1). It would be intriguing were these key life history decisions controlled by similar sets of molecular pathways in plants and animals, and recent data from Nonomura *et al.* [1] provide a glimpse into the molecular events that lead to meiosis and gametogenesis in plants. What emerges is evidence that, as in animals, proteins of the Argonaute family play a central role in plant 'germline' development.

The key genes involved in animal germline determination have been identified through mutagenesis and selection of phenotypes with reduced fertility. Significantly, the cell biology of germline cells is highly characteristic; for example, a common feature is the formation of diffuse, electron-opaque structures associated with the nucleus [2], termed 'nuage' in flies. Importantly, many of the proteins regulating germline development and gametogenesis are localised to the nuage, and several of these have been identified as RNA-associated proteins such as RNA helicases (for example, Vasa). These RNA-associated proteins also include members of the Argonaute family. Argonaute proteins are defined by two conserved domains named after its key members: the PAZ domain (PIWI, ARGONAUTE, ZWILLE) binds RNA, whilst the PIWI domain adopts an RNAse-like fold and has predicted endonuclease ('Slicer') activity. In *Drosophila*, Argonautes with known germline roles include the products of the *ago3, aubergine* and *piwi* genes [3,4]. In mice, the *MIWI*, *MILI* and *MIWI2* genes appear to be the functional homologues of *piwi* [4], despite the fact that flies and mice define their

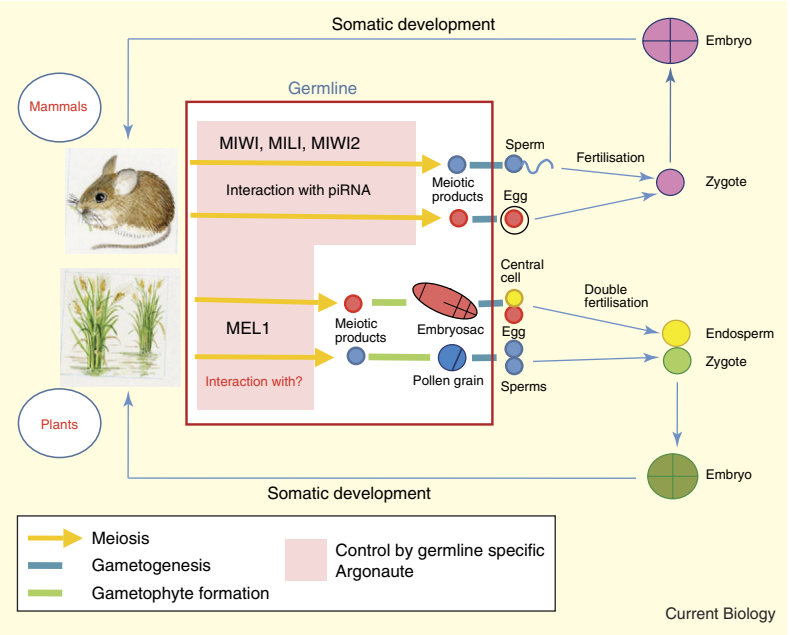


Figure 1. Activity of germline-specific Argonaute proteins during reproductive development of plants and animals.

Although plants form their gametes from gametophytes and have double fertilisation, the MEL1 Argonaute is active at exactly the same developmental stage as MIWI1/2 and MILI in mammals.

germlines in different ways: in flies the germline is 'preformed', while in mice it develops through 'epigenesis' [2].

How do these 'reproductive' Argonautes function to define germline identity? Dramatically, Argonautes of the Piwi subfamily have been shown to associate with a special germline-specific class of small RNAs, dubbed piRNAs. These small RNAs, identified in both fly and mammal germlines, are distinctive in their slightly larger size (24–31 nucleotides) and methylation [3,4]. Furthermore, they appear to be generated through a novel, Dicer-independent mechanism from substrates not used for making somatic small RNAs [3–6]. The piRNAs are abundant in the germline and homologous to both repetitive and single copy genomic sequences [7], and current evidence suggests that they direct chromatin changes to homologous genomic sites, modifying gene expression and 'protecting' the genome by silencing repetitive elements [8]. How these small RNAs effect chromatin changes remains undetermined. Interestingly, piRNAs do not have exclusive control of the germline, for there is accumulating evidence that microRNAs (miRNAs) play a significant role in reproductive development [9,10].

To date, plant Argonaute genes have almost exclusively been implicated in somatic development. Plants, however, do not have members of the PIWI subfamily, although the Argonaute gene family is significantly larger in plants than in animals, with 10 members in the small genome of *Arabidopsis* alone. Only ARGONAUTE1 has been implicated in post-meiotic reproductive development, as heterozygous *ago1* mutants transmit the mutant allele much less frequently than the wild-type allele [11]. ARGONAUTE1 was the first of the family to be discovered and is the main plant Slicer [12], responsible for the cleavage of target transcripts directed by miRNAs as well as short interfering (si)RNAs. Indeed, strong mutant *ago1* alleles disrupt development so severely in homozygotes that normal reproductive organs rarely form. In governing normal plant development, AGO1 functions in tandem with AGO10, also known as PINHEAD and ZWILLE [13], although the exact mechanism of AGO10 action remains to be clarified. Indeed, the function of half the family in *Arabidopsis* still remains undetermined.

Argonaute function has been most extensively investigated in *Arabidopsis*, although homologues with similar activities in other plant species have been discovered. The identification of a role for a novel Argonaute in rice [1] is, however, particularly exciting as — for the first time in plants — it suggests that an Argonaute has an essential and direct role in sexual reproduction. Nonomura *et al.* [1] identified seed-sterile mutant lines of rice that exhibited defective reproductive organogenesis and/or meiosis, but vegetative development remained normal. One of these mutant lines, named *mel1*, was found to disrupt the development of the cells destined to undergo meiosis in the anther — the so called 'sporogenous' cells. The cell biology of these cell lines also displayed subtle defects, for example, the sporogenous cells in the mutant contained mitochondria resembling those of the soma. In both male and female reproductive lineages, development was arrested over a range of stages extending from pre-meiosis, via meiosis itself to the immediately post-meiotic stages. Strikingly, where meiosis was arrested in *mel1* plants, the chromosomes remained uncondensed. When *MEL1* was cloned, it was found to encode an Argonaute, and after phylogenetic analysis designated as the founding member of a new plant-specific subfamily.

How does MEL1 operate? Neatly, *MEL1* transcripts were found only in the cells that will undergo meiosis, disappearing after meiosis. MEL1 somehow seems to mark these cells for a 'germline' fate, and evidence presented by Nonomura *et al.* [1] suggests that this is achieved through MEL1 acting on chromatin structure. This resonates with both the function of the Piwi subfamily in animal reproduction, and the discovery that two plant Argonautes, AGO4 and AGO6, play pivotal roles in RNA-mediated

DNA methylation and direct modification of the local chromatin structure [14–16]. It is proposed that the MEL1-imposed chromatin structure represses somatic gene expression programmes in these plant 'germ' cells.

The key question now remains as to what RNA species MEL1 interacts with, and how? We still have no information for plants as to whether entry and passage through the meiotic and gametogenic pathways involves reproduction-specific RNAs, equivalent to the animal piRNAs, or if stage-specific combinations of known RNA species (for example, miRNAs) carry out this function. The available data on non-coding RNAs in meiosis and gametogenesis are very sparse indeed, although recent work has shown that several miRNAs (such as *miR164* and *miR171*) are present in gametogenic cells and gametophytes [17] However, evidence for a complex RNA world regulating plant reproduction has started to emerge from a different direction, for recent work on *Arabidopsis* [18] has shown that a small gene family, the Arabidopsis-mei2-like (AML) RNA-binding proteins, has a major impact on controlling reproduction. Using a combination of RNA interference (RNAi) to down-regulate the five members of the gene family and a range of mutant alleles, Kaur *et al.* [18] showed that AMLs are required for both meiosis and gamete formation. However, the *AML* genes are also expressed in somatic growth, and their loss of function results in some vegetative phenotypes. As with MEL1, little is known about how these putative RNA-binding proteins operate *in vivo*, although it is intriguing that their closest relation, *mei2* in fission yeast, controls entry of cells into the meiotic programme [19]. In yeast, Mei2p is localised to the nucleus through its binding with meiRNA, a specific non-coding RNA species [20]. As with MEL1, Kaur *et al.* [18] suggest that AML proteins in plants translate RNA signals to affect, directly or indirectly, chromatin structure.

These new *MEL1* and *AML* data strongly highlight mechanistic similarities between plants, animals and yeasts in germline development (see Figure 1), but they also point to an interesting and important corollary — the apparent lability between somatic and reproductive cell lineages in plants. In rice, *MEL1* is one of several genes in its subfamily and *MEL1* is expressed solely in reproductive cells and does not function somatically; however, the single representative of the *MEL1* subfamily in *Arabidopsis,* AGO5 — for which a function has yet to be identified — is also expressed in vegetative as well as reproductive tissues. This discovery that *mei2* homologues have acquired a vegetative function in *Arabidopsis* indicates that the line between reproductive and somatic development in plants has become blurred, and serves as a molecular reminder of what is already appreciated by plant breeders — that plants easily switch between reproductive and somatic development programmes. For example, immature haploid gametophytes of plants can be induced to develop *in vitro* into new 'dihaploid' vegetative plants. Within the context of this recent work, it would now seem sensible to consider whether assumption of a reproductive or somatic cell fate ultimately hinges on epigenetic mechanisms such as RNA-directed chromatin changes.

## References

1. Nonomura, K.I., Morohoshi, A., Nakano, M., Eiguchi, M., Miyao, A., Hirochika, H., and Kurata, N. (2007). A germ cell specific gene of the ARGONAUTE Family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice. Plant Cell, In press.
2. Extavour, C.G., and Akam, M. (2003). Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. Development *130*, 5869–5884.
3. Lin, H. (2007). piRNAs in the germline. Science *316*, 397.
4. Seto, A.G., Kingston, R.E., and Lau, N.C. (2007). The coming of age for Piwi proteins. Mol. Cell *26*, 603.
5. Horwich, M.D., Li, C., Matranga, C., Vagin, V., Farley, G., Wang, P., and Zamore, P.D. (2007). The *Drosophila* RNA methyltransferase, DmHen1, modifies germline piRNAs and single-stranded siRNAs in RISC. Curr. Biol. *17*, 1265–1272.
6. Pane, A., Wehr, K., and Schupbach, T. (2007). *zucchini* and *squash* encode two putative nucleases required for rasiRNA production in the *Drosophila* germline. Dev. Cell. *12*, 851–862.
7. Aravin, A.A., Sachidanandam, R., Girard, A., Fejes-Toth, K., and Hannon, G.J. (2007). Developmentally regulated piRNA clusters implicate MILI in transposon control. Science *316*, 744–747.
8. Klenov, M.S., Lavrov, S.A., Stolyarenko, A.D., Ryazansky, S.S., Aravin, A.A., Tuschl, T., and Gvozdev, V.A. (2007). Repeat-associated siRNAs cause chromatin silencing of retrotransposons in the *Drosophila melanogaster* germline. Nucleic. Acids Res., In press.
9. Megosh, H.B., Cox, D.N., Campbell, C., and Lin, H. (2006). The role of PIWI and the miRNA machinery in *Drosophila* germline determination. Curr. Biol. *16*, 1884–1894.
10. Murchison, E., Stein, P., Xuan, Z., Pan, H., Zhang, M.Q., Schultz, R.M., and Hannon, G.J. (2007). Critical roles for Dicer in the female germline. Genes Dev. *21*, 682–693.
11. Kidner, C.A., and Martienssen, R.A. (2005). The role of ARGONAUTE1 (AGO1) in meristem formation and identity. Dev. Biol. *280*, 504–517.
12. Baumberger, N., and Baulcombe, D.C. (2005). Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc. Natl. Acad. Sci. USA *102*, 11928–11933.
13. Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., and Barton, M.K. (1999). The *PINHEAD/ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. Development *126*, 469–481.
14. Zilberman, D., Cao, X., and Jacobsen, S.E. (2003). *ARGONAUTE4* control of locus-specific siRNA accumulation and DNA and histone methylation. Science *299*, 716–719.
15. Qi, Y., He, X., Wang, X.-J., Kohany, O., Jurka, J., and Hannon, G.J. (2006). Distinct catalytic and non-catalytic role of ARGONAUTE4 in RNA-directed DNA methylation. Nature *443*, 1008–1012.
16. Zheng, X., Zhu, J., Kapoor, A., and Zhu, J.K. (2007). Role of Arabidopsis AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. EMBO J. *26*, 1691–1701.
17. Válóczi, A., Várallyay, É., Kauppinen, S., Burgyán, J., and Havelda, Z. (2006). Spatio-temporal accumulation of microRNAs is highly coordinated in developing plant tissues. Plant J. *47*, 140–151.
18. Kaur, J., Sebastian, J., and Siddiqi, I. (2006). The *Arabidopsis-mei2-like* genes play a role in meiosis and vegetative growth in *Arabidopsis*. Plant Cell *18*, 545–559.
19. Watanabe, Y., and Yamamoto, M. (1994). *S. pombe mei2+* encodes an RNA-binding protein essential for pre-meiotic DNA synthesis and meiosis I, which cooperates with a novel RNA species meiRNA. Cell *78*, 487–498.
20. Yamashita, A., Watanabe, Y., Nukina, N., and Yamamoto, M. (1998). RNA-assisted nuclear transport of the meiotic regulator Mei2p in fission yeast. Cell *95*, 115–123.

University of Oxford, Department of Plant Sciences, South Parks Road, Oxford OX1 3RB, UK.
E-mail: robert.grant-downton@plants.ox.ac.uk, hugh.dickinson@plants.ox.ac.uk

DOI: 10.1016/j.cub.2007.09.001